SUMMARY REPORT
OF THE EXTERNAL PEER REVIEW OF THE DRAFT TOXICOLOGICAL PROFILE FOR ACRYLAMIDE

Submitted to:
The Agency for Toxic Substances and Disease Registry
Division of Toxicology
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Submitted by:
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QUALITY NARRATIVE STATEMENT

ERG selected reviewers according to selection criteria provided by ATSDR. ATSDR confirmed that the scientific credentials of the reviewers proposed by ERG fulfilled ATSDR's selection criteria. Reviewers conducted the review according to a charge prepared by ATSDR and instructions prepared by ERG. ERG checked the reviewers' written comments to ensure that each reviewer had provided a substantial response to each charge question (or that the reviewer had indicated that any question[s] not responded to was outside the reviewer's area of expertise). Since this is an independent external review, ERG did not edit the reviewers' comments in any way, but rather transmitted them unaltered to ATSDR.

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SECTION A

PEER REVIEWERS' SUMMARY COMMENTS

SUMMARY COMMENTS RECEIVED FROM

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August 3, 2009

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U.S.A.

Peer Review of the
Draft (July 2009) Toxicological Profile for Acrylamide,
Prepared by SRC Inc. for ATSDR, USDHHS

I have carefully read the *Draft Toxicological Profile for Acrylamide* (here referred to as „Draft Profile"). This chemical has been discussed and reviewed within the last few years by a number of bodies in different countries, and regulatory actions are being considered.

In general, the toxicological properties of this chemical have been well investigated. Key points are neurotoxicity and carcinogenicity. There is an ongoing discussion in the scientific and regulatory arena of how to translate these properties and the associated modes of action into regulatory strategies. The current Draft Profile must be seen against this background.

The most recent published documentation on acrylamide is that of the Deutsche Forschungsgemeinschaft (The MAK-Collection Part I: MAK Value Documentations, Vol 25, Wiley-VCH, 2009). I attach a reprint of this documentation to this Review and refer to this as the "MAK Documentation".

In total, I find the Draft Profile well balanced and very well written. It presents the plethora of scientific results on this compound in a comprehensive way and is generally up to date.

There are a number of items, generally representing minor points that should be considered for the final revision of the Draft Review.

In the following, I follow the formal Guidelines for Peer Review of ATSDR's Toxicological Profiles.

Chapter 1. Public Health Statement

I have no specific comments regarding this chapter.

Chapter 2. Relevance to Public Health

I agree with the description of effects known to occur in humans, and with the way in which the animal data are presented. The derivations of NOAELs and/or LOAELs are in accordance with evaluations of other bodies internationally.

Chapter 3. Health Effects

Section 3.1 Introduction
This is standard text.

Section 3.2 Discussion of Health Effects by Route of Exposure

p.22, line 28: The term „muriatic acid" is not very common internationally. I would add in brackets „(hydrochloric acid)" to improve the understanding.

p.26, line12 („Endocrine Effects"): There is no mentioning of neurohormonal changes induced by acrylamide, which have been discussed in conjunction with the development of experimental tumours at some target sites (testicular, mammary tumours) – see the discussion and references on p. 36 of the MAK Documentation. This point should be re-considered in preparing the final version of the Profile.

p. 39, line 18ff („Neurological Effects“): It should be mentioned that in the case of the Norwegian tunnel workers, where signs of neurotoxicity were observed, dermal absorption was one way of exposure that has been discussed. A potential relevance of dermal absorption can also be inferred from the paper of Gutierrez-Espelata et al. (1992) [see references list of the Draft Profile].

Section 3.3 Genotoxicity

No comments

Section 3.4 Toxicokinetics

p.59, lines 1/2 (end of the chapter): This is difficult to understand (it should be more clearly explained).

Figure 3-6 is missing from the submitted set of „Revised Tables and Figures“. Therefore, I cannot understand what is meant by the „PBPK model units 1-4“ (p.58, lines 15/16).

Section 3.5 Mechanisms of Action

The state of discussion of mechanisms of toxicity is well presented for the neurotoxicity (p.59/69). By contrast, the discussion on mechanisms of carcinogenicity (p.61/62) is not as comprehensive. I recommend enlarging this section: elements of the respective discussion in the recent MAK Documentation (p.32ff) could eventually be incorporated.

Section 3.6 Toxicities Mediated through the Neuroendocrine Axis

No relevant data available – no comments.

Section 3.7 Children's Susceptibility

I agree with the conclusions of this chapter.

Section 3.8 Biomarkers of Exposure and Effect

There is one mistake: On p.68, l.30/31, it is said that the acrylonitrile and N-methylolacrylamide haemoglobin adducts cannot be distinguished from those of acrylamide. While this is true for N-methylolacrylamide (as shown in the biomonitoring studies from Scandinavia), the adducts from

acrylonitrile still carry the cyano group and are therefore clearly different from those derived from acrylamide! This must be corrected.

Section 3.9 Interactions with Other Chemicals

No comments.

Section 3.10 Populations that are Unusually Susceptible

I concur with the conclusions of this chapter.

Section 3.11 Methods for Reducing Toxic Effects

p.71, l. 34. Typo: It must be „*N*-Acetylcyteine“. (The initial N is just a chemical prefix. The word itself starts with the A.)

- The same typo appears also on p. 51, line 15.

Section 3.12 Existing Information on Health Effects of Acrylamide

p.73, l. 28-30: Although additional dermal studies would be fine, I personally do not support the plea for additional acute-duration studies, considering the entire existing database and arguments of animal welfare, and weighing the potential benefit of such additional studies.

Otherwise, the chapter appears well balanced.

Chapter 4. Chemical and Physical Information

This is not my primary field of expertise.

Chapter 5. Production, Import/Export, Use and Disposal

This is not my primary field of expertise.

Chapter 6 Potential for Human Exposure

I agree with the conclusions of this chapter.

Chapter 7 Analytical Methods

This is not my primary field of expertise.

Chapter 8 Regulations, Advisories and Guidelines

This is not my primary field of expertise.

Chapter 9. References

P. 117, l.12: The journal's name is missing from the reference Boettcher et al. (2006b). It is „Arch Toxicol".

Unpublished studies

All unpublished studies to which reference is made are industry-made or industry-sponsored studies, which were GLP-controlled or, in the pre-GLP time period, were conducted according to the state of the art at that time.

For an industrial bulk chemical like acrylamide, this is a normal situation. The way, in which the unpublished studies were used in the Draft Profile, is reasonable.

I have no suggestions for changes in this respect.

Dortmund, August 3, 2009

(Hermann M. Bolt, M.D., Ph.D.)

Professor emeritus of toxicology

SUMMARY COMMENTS RECEIVED FROM

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External Peer Review Comments
ATSDR's Toxicological Profile on Acrylamide

- ✓ *Are there any data relevant to child health and developmental effects that have not been discussed in the profile and should be?*

Yes

- ✓ *Are there any general issues relevant to child health that have not been discussed in the profile and should be?*

- ✓ *If you answer yes to either of the above questions, please provide any relevant references.*

Heudorf, U., Hartmann, E. and Angerer, J. (2009). Acrylamide in children--exposure assessment via urinary acrylamide metabolites as biomarkers. Int J Hyg Environ Health 212:135-41. Listed in the references but not cited or discussed.

CHAPTER 1. PUBLIC HEALTH STATEMENT

The intended audience for this chapter is the lay public, especially people living in the vicinity of a hazardous waste site or substance release. This chapter is written in active voice at an 8th to 10th grade reading level. To ensure that all relevant information has been incorporated, this chapter should be either reread after completing your review or, if only read once, read after reviewing the rest of the profile.

- *The tone of the chapter should be factual rather than judgmental. Does the chapter present the important information in a non-technical style suitable for the average citizen? If not, suggest alternate wording.*

The style and tone are appropriate.

- *Major headings are stated as a question. In your opinion, do the answers to the questions adequately address the concerns of the lay public? Are these summary statements consistent, and are they supported by the technical discussion in the remainder of the text? Please note sections that are weak and suggest ways to improve them.*

Some of the answers are not adequately supported or consistent with reading supporting material. See comments below.

- *Are scientific terms used that are too technical or that require additional explanation? Please note such terms and suggest alternate wording.*

No.

Page 1, line 25. A general point that requires clarification is the extent to which acrylamide is used in water treatment. My understanding of its main use is not in water treatment in the preparation of drinking water, but rather the recovery of water in industrial process, e.g. in removing water from sludge, e.g. in sewage treatment. Haberman et al (2002) indicates that "the largest use for this category is as a dewatering aid for sludges in the treatment of effluent from municipal wastewater treatment plants, and industrial processes".

Page 2, section 1.2. Most commonly found in water.

Page 2, section 1.3, line 5? The table row on water and soil cites a paper by Cavallli et al. (2004), and HSDB 2009. In trying to track the source of the information on exposure if you live near a plastic or dye plant, it would appear that the Cavalli paper is a methods paper on the analysis of acrylamide spiked into water, and indicates that "Polyacrylamides are also used as grouting agents in the construction of drinking water reservoirs and wells, and can be discharged in land and water by plastics and dyes industries". No specific citation is provided for this information, and the paragraph in which the information occurs cites

Risk Assessment of Acrylamide, Draft, European Union, Brussels, October 2000, p. 283, as a source of "…all the available information about production, use, fate, and toxicological properties."

The paper by Cavalli et al. (2004) is not an authoritative source on exposure near a plastic and dye plant, and is used in <u>many</u> places throughout this text is support of the assertion of exposure near a plastic or dye plant. It should be replaced with an appropriate citation, and checked for accuracy of the information.

CHAPTER 2. RELEVANCE TO PUBLIC HEALTH

The purpose of this section is to evaluate and interpret the significance of existing toxicity data and, in some cases, speculate regarding the significance of this information as it relates to human health. Specifically, the text should address what effects are known to occur in humans; what effects have been observed in animals but not in humans; and what exposure conditions (route, duration, or level) are likely to be of concern to humans, especially around hazardous waste sites?

- *Do you agree with those effects known to occur in humans as reported in the text? If not, provide a copy of additional references you would cite and indicate where (in the text) these references should be included.*

The neurological effects in people are correctly identified.

- *Are the effects only observed in animals likely to be of concern to humans? Why or why not? If you do not agree, please explain.*

I agree with the selection of neurological effects, cancer, and reproductive toxicity. The relevance of body weight gain as a serious toxic endpoint was less obvious.

- *Have exposure conditions been adequately described? If you do not agree, please explain.*

See points requiring clarification:

Page 8, line 10. The citation of American Cyanamid Company, 1991 is not appropriate in this instance. The reference on page 113, line 30 is to a 28-day sub-chronic toxicity study in male and female rats, and not a dominant lethal study.

Page 9, lines 5 and 8. EPA (2009). Which one? There are 6 (a-f) in the References on page 124.

Page 9, line 17. The description of body weight effects following a single dose should be revisited. See comments on the report Dow Chemical Company 1957, in which there are response remarks included in the tables, with such comments as "slight initial weight loss", or "very slight initial weight loss". However, no details of the number of animals affected, the extent of the weight loss, or whether it was statistically significant were reported. Is this information reported in McCollister et al., 1964?
Page 9, line 17-19. The study of Tyl et al. 2000b did not report a 43% depressed body weight gain with dosing of 15 mg/kg/day for 5 days. At 15 mg/kg/day, the effect is just significant.

Page 9, line 19-21. The reference to Post and McLeod (1977a) refers to cats. A reference is needed for dogs.

Page 11, line 22 – 24. How was the dose administered in the Burek et al. study? Drinking water? This is in contrast with the Tyl et al. 2000b study, where the dose was administered by daily gavage.

Page 11, line 31. The document reports that "a 43% depressed body weight gain was reported following oral administration of acrylamide at 15 mg/kg/day for 5 days; this effect was not observed at 5 mg/kg/day (Tyl et al. 2000b)." Review of Tyl et al. (2000b) indicated that the number of 43% was prepared by the reviewer, and it was not immediately obvious what this meant, and has the potential for confusion. It appears that male body weight gain was statistically significantly reduced at 15, 30, 45 and 60 mg/kg/day

for 5 days. However, the change reported at 15 mg/kg/day was approximately a body weight gain of 13 g, compared with a body weight gain in the control group of 22 g (values were extracted from Figure 1 of the paper), which corresponds to a weight gain in the 15 mg/kg/day of approximately 60% of the control value. Administration of higher doses did result in weight loss (approximately 2, 16, and 37 g at 30, 45, and 60 mg/kg/day, respectively).

Page 12, line 9. Note the comments above concerning the Tyl et al. (2000b) study. The decreased body weight gains (>40%) were observed at 15 mg/kg/day or above.

CHAPTER 3. HEALTH EFFECTS

The intended audience for this chapter includes community-level public health officials, physicians, and concerned citizens. It is not intended to be a data review for toxicologists. Emphasis is placed on providing a summary evaluation of the weight of evidence, rather than on providing detailed descriptions of every relevant study. Scientifically prudent judgments and interpretations are both appropriate and desirable.

Section 3.1 INTRODUCTION

This introduction is standard language (in bold). A brief substance-specific discussion may be added to explain a complex topic.

Section 3.2 DISCUSSION OF HEALTH EFFECTS BY ROUTE OF EXPOSURE

This section begins with standard language (in bold). The purpose of this section is to specify the health effects that are associated with the substance and the degree of certainty attached to that association. Negative data also are presented. The text should contain conclusions about whether the effect occurs or not and about whether the studies are reliable. Human data should be presented before animal data. When information suggests that an effect occurs, but the dose/response relationship is unclear, the issue should be discussed in the text.

In this section, toxicological effects are organized according to route of exposure (inhalation, oral, and dermal). Most of the information describing reliable studies is presented in the levels of significant exposure (LSE) tables. Text should be reserved for conclusions, discussions, explanations, etc. ***NOTE****: Other routes of exposure (e.g., intraperitoneal, intramuscular, or subcutaneous) and <u>in vitro</u> studies are not discussed here; this information is included in Chapter 2: Relevance to Public Health.*

ATSDR follows the National Research Council's "Guidelines for Assessing the Quality of Individual Studies," in <u>Toxicity Testing: Strategies to Determine Needs and Priorities</u> (NRC 1984).

Toxicity - Quality of Human Studies

- *Were adequately designed human studies identified in the text (i.e., good exposure data, sufficiently long period of exposure to account for observed health effects, adequate control for confounding factors)? If not, were the major limitations of the studies sufficiently*

described in the text without providing detailed discussions. If study limitations were not adequately addressed, please suggest appropriate changes.

Yes.

- *Were the conclusions drawn by the authors of the studies appropriate and accurately reflected in the profile? If not, did the text provide adequate justification for including the study (e.g., citing study limitations)? Please suggest appropriate changes.*

Yes

- *Were all appropriate NOAELs and/or LOAELs identified for each study? If not, did the text provide adequate justification for excluding NOAELs/LOAELs including, but not limited to, citing study limitations? Please suggest appropriate changes.*

- *Were the appropriate statistical tests used in the studies? Would other statistical tests have been more appropriate? Were statistical test results of study data evaluated properly? NOTE: As a rule, statistical values are not reported in the text, but proper statistical analyses contribute to the reliability of the data.*

- *Are you aware of other studies which may be important in evaluating the toxicity of the substance? Please provide a copy of each study and indicate where in the text each study should be included.*

Toxicity - Quality of Animal Studies

- *Were adequately designed animal studies identified in the text (i.e., adequate number of animals, good animal care, accounting for competing causes of death, sufficient number of dose groups, and sufficient magnitude of dose levels)? If not, does the inadequate design negate the utility of the study? Please explain.*

- *Were the animal species appropriate for the most significant toxicological endpoint of the study? If not, which animal species would be more appropriate and why?*

- *Were the conclusions drawn by the authors of the studies appropriate and accurately reflected in the text? If not, did the text provide adequate justification for including the study (e.g., citing study limitations)?*

- *Were all appropriate NOAELs and LOAELs identified for each study? Were all appropriate toxicological effects identified for the studies? If not, please explain.*

- *If appropriate, is there a discussion of the toxicities of the various forms of the substance? If not, please give examples of toxicological effects that might be important for forms of the substance.*

- *Were the appropriate statistical tests used in the interpretation of the studies? If not, which statistical tests would have been more appropriate? Were statistical test results of study data evaluated properly? **NOTE**: As a rule, statistical values are not reported in the text, but proper statistical analyses contribute to the reliability of the data.*

- *Are you aware of other studies that may be important in evaluating the toxicity of the substance? If you are citing a new reference, please provide a copy and indicate where (in the text) it should be included.*

Page 23, line 22, units are required for the air concentrations.

Levels of Significant Exposure (LSE) Tables and Figures

These tables and figures are used to summarize health effects and graphically illustrate levels of exposure associated with those effects. These tables and figures present information on health effects by route, duration, increasing dose concentration, differences in response by species, minimal risk levels (MRLs) to humans for noncancer endpoints, cancer effect levels (CELs), and EPA's estimated range associated with an upper-bound cancer risk of 1 in 10,000 to 1 in 10,000,000.

*All studies that are identified in the text **are not** presented in the LSE tables and figures. Studies that lack quantitative estimates of NOAELs and LOAELs, or that are not reliable, should not be selected for inclusion. All data in an LSE table must be plotted on the corresponding LSE figure, with the exception that dermal data are presented in an LSE table without an accompanying LSE figure. For a description of MRLs and how to use the LSE tables and figures, see the "User's Guide" in the profile.*

- *Are the LSE tables and figures complete and self-explanatory? Does the "Users Guide" explain clearly how to use them? Are exposure levels (units, dose) accurately presented for the route of exposure? Please offer suggestions to improve the effectiveness of the LSE tables and figures and the "User's Guide."*

- *Do you agree with the categorization of "less serious" or "serious" for the effects cited in the LSE tables?*

- *If MRLs have been derived, are the values justifiable? If no MRLs have been derived, do you agree that the data do not support such a derivation?*

Comments related to LSE Tales. Are there values that are repeated from different citations but represent the same data, e.g. Table 3-4, items 13 and 14?

To help the reviewer, the information should be provided electronically as well as in hard copy. This would greatly facilitate searching and cross referencing.

Evaluation of Text

- *Have the major limitations of the studies been adequately and accurately discussed? How might discussions be changed to improve or more accurately reflect the proper interpretation of the studies?*

- *Has the effect, or key endpoint, been critically evaluated for its relevance in both humans and animals?*

- *Have "bottom-line" statements been made regarding the relevance of the endpoint for human health?*

- *Are the conclusions appropriate given the overall database? If not, please discuss your own conclusions based on the data provided and other data provided to you but not presented in the text.*

- *Has adequate attention been paid to dose-response relationships for both human and animal data? Please explain.*

- *Has the animal data been used to draw support for any known human effects? If so, critique the validity of the support.*

In answer to all these questions, there are some instances where the text adequately describes the study design, the limitations and the conclusions. However, there are instances where the writers are comparing studies where there are key differences that might be expected to cause differences. See for example, page 31, lines 5-20. On lines 8-10, the review describes the study of Sublet et al (1989) reporting decreased sperm motility, but suggesting that this effect was not solely responsible for poor reproductive performance. Tyl et al (2000b) followed up on this study, and suggested that there may be a component of neurotoxicity involved.

This paragraph also discusses testicular atrophy following acrylamide administration in the drinking water for 28 days or 90 days, at concentrations of 19 or 5 mg/kg/day, but in apparent contrast the lack of findings of Tyl et al. (2000b), who reported no significant effect on sperm parameters following repeated exposure to dose levels as high as 60 mg/kg. It is worth remembering that the duration of dosing in the Tyl study was 5 days, not 28 or 90 days.

Section 3.3 GENOTOXICITY

No comments.

Section 3.4 TOXICOKINETICS

This section, like all preceding sections, should provide a synthesis and a weight-of-evidence analysis of toxicokinetics without detailed descriptions of individual studies (unless they are key to understanding the data). ***[p. 48 of guidance states "with a description and discussion of key studies"]*** *Special attention should be focused on significant toxicokinetic differences between high- vs. low-level exposure and sex or species differences (especially between humans and animals) that might be relevant in extrapolation of animal toxicity data to humans. As in the discussion of toxicological effects, the section should be organized by human vs. animal studies and, within these, by duration of exposure where possible.*

- *Is there adequate discussion of absorption, distribution, metabolism, and excretion of the substance? If not, suggest ways to improve the text.*

Hemoglobin adducts have provided a way to monitor exposure and to estimate internal dose of both acrylamide and its reactive metabolite glycidamide. A more detailed discussion of the formation of adducts, and what is being measured as an introduction to section 3.4 would improve the text.

- *Have the major organs, tissues, etc. in which the substance is stored been identified? If not, suggest ways to improve the text.*

- *Have all applicable metabolic parameters been presented? Have all available pharmacokinetic/pharmacodynamic models and supporting data been presented? If not, please explain.*

Not all pharmacokinetic models have been described. A PB-PK model based on the Kirman model was published recently by Walker, K., Hattis, D., Russ, A., Sonawane, B. and Ginsberg, G. (2007). Approaches to acrylamide physiologically based toxicokinetic modeling for exploring child-adult dosimetry differences. J Toxicol Environ Health A 70:2033-55.

A pharmacokinetic model describing the non-linearity of glycidamide formation as a function of administered dose was reported by Calleman et al. (1992, 1993).

Calleman, C. J., Stern, L. G., Bergmark, E. and Costa, L. G. (1992). Linear versus nonlinear models for hemoglobin adduct formation by acrylamide and its metabolite glycidamide: implications for risk estimation. Cancer Epidemiol Biomarkers Prev 1:361-8.

Calleman, C. J., Bergmark, E., Stern, L. G. and Costa, L. G. (1993). A nonlinear dosimetric model for hemoglobin adduct formation by the neurotoxic agent acrylamide and its genotoxic metabolite glycidamide. Environ Health Perspect 99:221-3.

- *Is there adequate discussion of the differences in toxicokinetics between humans and animals? What other observations should be made?*

Although there is extensive presentation of data from different species, there is little discussion of species differences in toxicokinetics in Section 3.4 Toxicokinetics. This could be improved by a more extensive comparison of the metabolism of acrylamide to glycidamide as reflected in hemoglobin adducts, and species differences in the relative ratios of acrylamide-valine to glycidamide-valine.

- *Is there an adequate discussion of the relevance of animal toxicokinetic information for humans? If not, please explain.*

- *If applicable, is there a discussion of the toxicokinetics of different forms of the substance (e.g., inorganic vs. organic mercury)?*

Comments related to Toxicokinetics

Page 44, lines 22-24. The position and nature of the isotope labels is important, and should be included. 1,2,3-$^{13}C_3$ acrylamide is a stable isotope labeled form with approximately 99% enrichment at each carbon. It was administered to enrich the ^{13}C NMR signals of the metabolites of acrylamide in urine, to provide a means of detecting all of the metabolites of acrylamide. In addition or instead, depending on the specific exposure, [2,3-^{14}C] acrylamide, which is a radioactive label, enriched by a small fraction, was used to track the disposition of acrylamide. In some instances these were used separately, and in some, a small percentage of [2,3-^{14}C] and a high percentage of 1,2,3-$^{13}C_3$ acrylamide were mixed, and used to track disposition by measurement of radioactivity, and qualitative and quantitative analysis of metabolites was conducted by ^{13}C NMR spectroscopy.

Page 45, section 3.4.1.2 Oral Exposure, lines 6-27. This paragraph appears under the heading of Oral Exposure, and the study described (Fennell et al. 2005b) did indeed have a substantial focus on oral exposure. However, much of the paragraph is focused on a separate dermal component of the study, rather than on the important details of the oral study. A follow up component of this study is not mentioned (Fennell et al, 2006), in which the urine samples were analysed for the kinetics of elimination of a urinary metabolites by liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS). The products analyzed included acrylamide, glycidamide, and N-acetylcysteine conjugates of acrylamide and glycidamide, as well as a new characterized N-acetylcysteine sulfoxide conjugate of acrylamide.

This product is isomeric with N-acetylcysteine conjugates of glycidamide, and care is needed in the analysis of these products by LC-MS.

Fennell, T. R., Sumner, S. C., Snyder, R. W., Burgess, J. and Friedman, M. A. (2006). Kinetics of elimination of urinary metabolites of acrylamide in humans. Toxicol Sci **93**:256-67.

Boettcher et al. (2006a), which is included in the reference list with an asterisk, should be cited in Section 3.4.1.2 Oral Exposure, page 45-47. The study is described later in Metabolism.

Page 47, line 28. The text notes that animal studies confirm that acrylamide is readily absorbed following dermal exposure. There are also dermal studies in humans indicating uptake measured by hemoglobin adducts (Fennell et al. 2005b), and urinary metabolites (Fennell et al. 2006).

Fennell, T. R., Sumner, S. C., Snyder, R. W., Burgess, J. and Friedman, M. A. (2006). Kinetics of elimination of urinary metabolites of acrylamide in humans. Toxicol Sci 93:256-67.

Page 48, line 32 – Page 49 line 2. This section on inhalation exposure has no reference to the source of the data presented. Is it Sumner et al. (2003)?

Page 50, line 7. There is a discrepancy between the citations for the source of Figure 3-3 in the text and in the Figure. Sumner et al. 1992 is missing from the Figure.

Page 51, line 16. The position and nature of the radiolabel should be specified: [2,3-^{14}C].

Page 51, line 17. The material used by Sumner et al. was labeled with ^{13}C, and thus is not radiolabeled. It was 1,2,3-$^{13}C_3$ acrylamide.

Page 51, lines 28-page 52, line 7. An important consideration of the release of $^{14}CO_2$ is the position of radiolabel in the administered acrylamide. It is not possible to interpret the studies described in this paragraph without this key piece of information in each study. Hasimoto and Aldridge (1970) administered [1-^{14}C] acrylamide and detected 6% as $^{14}CO_2$, whereas Miller et al. (1982) administered [2,3-^{14}C] acrylamide, and detected no $^{14}CO_2$. Kadry et al. (1999) used [1-^{14}C] acrylamide, and found no $^{14}CO_2$. Ramsey et al. (1984) used [1,3-^{14}C], and detected 3-4% as $^{14}CO_2$. It appears that the presence of ^{14}C in the 1-position is required for detection of $^{14}CO_2$, although the Kadry et al. study indicates that CO_2 is not released in all cases.

Page 52, line 9 – Page 54, line 7. This portion of the text on metabolism describes studies in humans. There is merit in ordering the studies in chronological order, and not in alphabetical order of the first authors. Some differences in approach of the three studies are worth noting. The Fennell et al. (2005b) study used a 1,2,3-$^{13}C_3$ label to investigate the metabolism of the administered acrylamide by ^{13}C NMR spectroscopy, with no preconceptions of the nature of the metabolites. Acrylamide, glycidamide, glyceramide, and N-acetyl-S-(2-carbamoyl-2-hydroxyethyl)cysteine were detected and quantitated, together with N-acetyl-S-(2-carbamoylethyl)cysteine and its sulfoxide, a new metabolite. In contrast, in the Boettcher et al. (2006a) study, deuterium-labeled acrylamide was administered, and deuterium-labeled N-acetyl-S-(2-carbamoylethyl)cysteine (AAMA) and N-acetyl-S-(2-carbamoyl-2-hydroxyethyl)cysteine (GAMA) were detected by LC-MS. Thus, this study specifically targeted two metabolites. The Fuhr et al (2006) study did not use labeled acrylamide, and targeted acrylamide, glycidamide, AAMA and GAMA for analysis using LC-MS. The Fennell et al. (2006) study which is not included in the description expands on the Fennell et al. (2005) study, and includes analysis of urinary metabolites by LC-MS/MS at three dose levels following oral administration, and following repeated dermal administration.

Page 54, line 14. Fennell et al. (2006) and Sumner et al. (2003) should be added to the citations.

Page 54, line 18. "No human or animal data were located regarding excretion and elimination following inhalation exposure to acrylamide." There is one study on the inhalation exposure of rats and mice to acrylamide and its metabolism reported in Sumner et al. (2003).

Page 55, line 29. The text indicates that "no human or animal data were located regarding excretion and elimination following dermal exposure to acrylamide". Fennell et al. (2006) described excretion of acrylamide and its metabolites following dermal administration in humans and Sumner et al. (2003) reported excretion in urine of acrylamide administered dermally to rats and mice.

Figure 3-3. Metabolism of acrylamide and glycidamide. There is a metabolite missing: the acrylamide mercapturic acid sulfoxide, described by Fennell et al. 2005, 2006. The CH2 and NH2 throughout should be subscripted.

In addition, it is important to note that the metabolite glyceramide has only been quantitated in human urine by NMR spectroscopy in combination with administration of 1,2,3-$^{13}C_3$ acrylamide. No other assay for this compound has been published.

Section 3.5 MECHANISMS OF ACTION

The propose of this section is to provide a brief overview of known mechanisms of metabolism, absorption, distribution, and excretion, and then a discussion of any substance reactions or physiological processes that may affect these mechanisms. Have all possible mechanisms of action been discussed? If not, please explain.

One of the key questions in the toxicities associated with acrylamide is whether acrylamide mediates the activity, or if metabolism to glycidamide is required. Page 59. section 3.5.1 lines 12-14 notes that both parent compound and the epxoide glycidamide appear to be involved in acrylamide toxicity, and that animal data note demonstrate the importance of CYP2E1 in acrylamide metabolism. Elaboration on several factors would enhance this discussion: the proportion of acrylamide metabolized to glycidamide, and the relative area under the curve for glycidamide relative to acrylamide is dose-, route-, and species-dependant. Understanding whether acrylamide or glycidamide is involved in the mode of action of a particular toxicity could help in understanding the potential risk to humans from exposure to acrylamide. The demonstration that CYP2E1 is required for metabolism of acrylamide to glycidamide in mice, and the absence of glycidamide production in CYP2E1 null mice provides a tool for dissecting the role of glycidamide in acrylamide toxicity. It would be helpful to add work by Ghanayem and colleagues to the discussion of mechanisms of toxicity, and also the mechanism of genotoxicity (Page 61, line 31 – Page 62, line 12):

Ghanayem, B. I., McDaniel, L. P., Churchwell, M. I., Twaddle, N. C., Snyder, R., Fennell, T. R. and Doerge, D. R. (2005). Role of CYP2E1 in the epoxidation of acrylamide to glycidamide and formation of DNA and hemoglobin adducts. Toxicol Sci 88:311-8.

Ghanayem, B. I., Witt, K. L., El-Hadri, L., Hoffler, U., Kissling, G. E., Shelby, M. D. and Bishop, J. B. (2005). Comparison of germ cell mutagenicity in male CYP2E1-null and wild-type mice treated with acrylamide: evidence supporting a glycidamide-mediated effect. Biol Reprod 72:157-63.

Ghanayem, B. I., Witt, K. L., Kissling, G. E., Tice, R. R. and Recio, L. (2005). Absence of acrylamide-induced genotoxicity in CYP2E1-null mice: evidence consistent with a glycidamide-mediated effect. Mutat Res 578:284-97.

Section 3.6 TOXICITIES MEDIATED THROUGH THE NEUROENDOCRINE AXIS

Section 3.7 CHILDREN'S SUSCEPTIBILITY

Page 66, line 30. "No studies were located that examined levels of acrylamide in breast milk from human or animal mothers exposed to acrylamide". The study by Sorgel et al. (2002) cited elsewhere in this document describes the measurement of acrylamide in human breast milk.

Sorgel, F., Weissenbacher, R., Kinzig-Schippers, M., Hofmann, A., Illauer, M., Skott, A. and Landersdorfer, C. (2002). Acrylamide: increased concentrations in homemade food and first evidence of its variable absorption from food, variable metabolism and placental and breast milk transfer in humans. Chemotherapy 48:267-74.

Heudorf, U., Hartmann, E. and Angerer, J. (2009). Acrylamide in children--exposure assessment via urinary acrylamide metabolites as biomarkers. Int J Hyg Environ Health 212:135-41. Listed in the references but not cited or discussed.

Section 3.8 BIOMARKERS OF EXPOSURE AND EFFECT

This section begins with standard language (in bold).

- *Are the biomarkers of exposure specific for the substance or are they for a class of substances? If they are not specific, how would you change the text?*

Metabolites can be measured for assessment of short term exposure. Hemoglobin adducts can be measured for the assessment of longer term exposure.

Page 68. The statement on lines 30-31 is only partly correct. N-Methylolacrylamide forms adducts that are similar to acrylamide. However, with acrylonitrile, it depends on what method of analysis is used for adduct analysis. If cysteine adducts are used with hydrolysis of globin, and analysis of S-(2-carboxyethyl)cysteine released, then the same product is formed by acrylonitrile and acrylamide adducts. However, if valine adducts are analyzed using the modified Edman degradation method, then there is no interference, since cyanoethylvaline is formed with acrylonitrile, and it can readily be distinguished from the acrylamide-valine adduct.

- *Are there valid tests to measure the biomarker of exposure? Is this consistent with statements made in other sections of the text? If not, please indicate where inconsistencies exist.*

There are valid measures and have been described by a number of investigators.

- *Are the biomarkers of effect specific for the substance or are they for a class of substances? If they are not specific, how would you change the text?*

With the exception of glycidamide-DNA adducts, biomarkers of effect are not specific for exposure to acrylamide.

- *Are there valid tests to measure the biomarker of effect? Is this consistent with statements made in other sections of the text? If not, please indicate where inconsistencies exist.*

Page 68, lines 20 -25. The description of urinary metabolites is missing a key study: Fennell et al. (2006).

Fennell, T. R., Sumner, S. C., Snyder, R. W., Burgess, J. and Friedman, M. A. (2006). Kinetics of elimination of urinary metabolites of acrylamide in humans. Toxicol Sci 93:256-67.

Section 3.9 INTERACTIONS WITH OTHER CHEMICALS

Discuss the influence of other substances on the toxicity of the substance.

- *Is there adequate discussion of the interactive effects with other substances? Does the discussion concentrate on those effects that might occur at hazardous waste sites? If not, please clarify and add additional references.*

- *If interactive effects with other substances are known, does the text discuss the mechanisms of these interactions? If not, please clarify and provide any appropriate references.*

Section 3.10 POPULATIONS THAT ARE UNUSUALLY SUSCEPTIBLE

This section begins with standard language (in bold) and identifies known or potential unusually-susceptible populations.

- *Is there a discussion of populations at higher risk because of biological differences which make them more susceptible? Do you agree with the choices of populations? Why or why not? Are you aware of additional studies in this area?*

Section 3.11 METHODS FOR REDUCING TOXIC EFFECTS

Where data or reasonable conjecture permit, this section describes directions of clinical practice and research that may help develop new methods for reducing toxic effects in individuals or populations exposed to a substance. It is intended to inform the public of existing clinical practice(s) and the status of research concerning such methods. It is not intended as a guide to treatment for poisoning.

When possible, a distinction should be made between differences in management and treatment following acute (generally high-level) vs. chronic (generally low-level) exposure. The section should

not include dosages nor detailed descriptions of treatment regimens. The section should not read as though ATSDR is endorsing or recommending any particular treatment.

*The first part of the section should be brief and provide a **very general** discussion regarding treatments that are known or expected to reduce peak absorption (lower initial blood levels) of the substance following exposure.*

- *Is the management and treatment specific for the substance, or is it general for a class of substances?*
- *Is there any controversy associated with the treatment? Is it a "well-accepted" treatment?*
- *Are there any hazards associated with the treatment of populations that are unusually susceptible to the substance (e.g., infants, children)?*

The second part of the section should concentrate on methods to enhance the elimination of the absorbed dose or body burden, or remove a persisting metabolite or by-product of the substance from the body. It is appropriate to discuss treatments or research regarding interference with mechanisms of distribution or retention, or alteration of the pharmacokinetics of the substance so it has less chance of reaching the target organ(s).

- *Are treatments available to prevent the specific substance from reaching the target organ(s), or are the actions general for a class of substances?*
- *Is there any controversy associated with the treatment?Is it a "well-accepted" treatment? If the discussion concerns an experimental method, do you agree with the conceptual approach of the method?*
- *Are there any hazards associated with the treatment of populations that are unusually susceptible to the substance (e.g., infants, children)?*
- *Are there treatments to prevent adverse effects as the substance is being eliminated from the major organs/tissues where it has been stored (e.g., as a substance is eliminated from adipose tissue, can we prevent adverse effects from occurring in the target organ[s])?*

The last part of the section should focus on clinical or experimental methods that are known or expected to block the mechanism of toxic action at any point from initial interaction with body processes, to the actual physical damage or functional change.

- *Are treatments available to prevent the specific substance from reaching the target organ(s), or are the treatment's actions general for a class of substances?*
- *Is there any controversy associated with the treatment? Is it a "well-accepted" treatment? If the discussion concerns an experimental method, do you agree with the conceptual approach of the method?*
- *Are there any hazards associated with the treatment of populations that are unusually susceptible to the substance (e.g., infants, children)?*

Section 3.12 ADEQUACY OF THE DATABASE

This section begins with standard ATSDR language (in bold). "Data needs" are defined as substance-specific informational needs that, if met, would reduce or eliminate the uncertainties of human health assessment. This definition should not be interpreted to mean that all data needs discussed in this section must be filled. In the future, the identified data needs will be evaluated and prioritized and a substance-specific research agenda will be proposed.

Existing Information on Health Effects of [Substance X]

Figure 2-X "Existing Information on Health Effects of [Substance X]" is provided to illustrate that positive and negative data exist. There is standard language (in bold) in the text. The dots in the figure do not imply anything about the quality of the study or studies. Gaps in this figure should not be interpreted as "data needs" information.

- *Do you know of other studies that may fill a data gap? f so, please provide the reference.*

Identification of Data Needs

Carefully consider the data needs because they will serve as the basis for establishing a substance-specific research agenda. Data needs are discussed in Sections 6.8.1, 6.8.2 and 7.3.1 as well. The following questions also pertain to both of those sections.

- *Are the data needs presented in a neutral, non-judgmental fashion? Please note where the text shows bias.*

Yes.

- *Do you agree with the identified data needs? If not, please explain your response and support your conclusions with appropriate references.*

In general yes. I would like to see a method developed for the analysis of glyceramide in urine as an additional metabolite marker to assess the relative importance of metabolism of glycidamide via glutathione conjugation vs. hydrolysis. Also there is no clear understanding of the role of enzymes in the metabolism of acrylamide and glycidamide via glutathione conjugation (is it enzyme-mediated, or spontaneous?). Similarly, is hydrolysis of glycidamide spontaneous and acid or base catalyzed in vivo, or is it enzyme mediated? An investigation of the role of enzymes in the metabolism of acrylamide would improve the development of PB-PK models.

It is generally thought that genotoxicity is mediated by glycidamide. An understanding of the role of glycidamide vs. acrylamide in other actions such as neurotoxicity is desirable. Glycidamide adducts are widely formed in the body, yet tumors are formed in a few organs. The potential role for other effects of acrylamide and or glycidamide exposure in causing the development of tumors is needed.

- *Does the text indicate whether any information on the data need exists?*

Yes

- *Does the text adequately justify why further development of the data need would be desirable; or, conversely, justify the "inappropriateness" of developing the data need at present? If not, how can this justification be improved.*

Yes

Page 77, line 19. The citation of American Cyanamid Company, 1991 is not appropriate in this instance. The reference on page 113, line 30 is to a 28-day sub-chronic toxicity study in male and female rats, and not a dominant lethal study.

Page 79, line 29 – 31. Same comment as before concerning specificity of hemoglobin adducts: The statement on lines 30-31 is only partly correct. N-Methylolacrylamide forms adducts that are similar to acrylamide. However, with acrylonitrile, it depends on what method of analysis is used for adduct analysis. If cysteine adducts are used with hydrolysis of globin, and analysis of S-(2-carboxyethyl)cysteine released, then the same product is formed by acrylonitrile and acrylamide adducts. However, if valine adducts are analyzed using the modified Edman degradation method, then there is no interference, since cyanoethylvaline is formed with acrylonitrile, and it can readily be distinguished from the acrylamide-valine adduct. In addition, for most studies on exposure to acrylamide, co-exposure to N-methylolacrylamide could be accounted for.

CHAPTER 4. CHEMICAL AND PHYSICAL INFORMATION

This chapter should contain very little text. Most of the information should be presented in tabular form.

- *Are you aware of any information or values that are wrong or missing in the chemical and physical properties tables? Please provide appropriate references for your additions or changes.*

No.

Is information provided on the various forms of the substance? If not, please explain.

CHAPTER 5. PRODUCTION, IMPORT/EXPORT, USE, AND DISPOSAL

The level of detail in this chapter should be appropriate to an overview.

- *Are you aware of any information that is wrong or missing? If so, please provide copies of the references and indicate where (in the text) the references should be included.*

No.

CHAPTER 6. POTENTIAL FOR HUMAN EXPOSURE

This chapter includes general statements describing the ways in which substance releases are modified by time and environmental fate processes and the potential for human exposure to the substance via the different pathways.

- *Has the text appropriately traced the substance from its point of release to the environment until it reaches the receptor population? Does the text provide sufficient and technically sound information regarding the extent of occurrence at NPL sites? Do you know of other relevant information? Please provide references for added information.*

While the formulation in cosmetics and consumer products is noted on page 87, line 18, there is no discussion of the potential for dermal exposure to residual acrylamide monomer in cosmetics and consumer products.

- *Does the text cover pertinent information relative to transport, partitioning, transformation, and degradation of the substance in all media? Do you know of other relevant information? Please provide references for added information.*

- *Does the text provide information on levels monitored or estimated in the environment, including background levels? Are proper units used for each medium? Does the information include the form of the substance measured? Is there an adequate discussion of the quality of the information? Do you know of other relevant information? Please provide references for added information.*

- *Does the text describe sources and pathways of exposure for the general population and occupations involved in the handling of the substance, as well as populations with potentially high exposures? Do you agree with the selection of these populations? If not, why? Which additional populations should be included in this section?*

- *For Sections 6.8.1, Identification of Data Needs and 6.8.2, Ongoing Studies, answer the same questions presented in Section 3.12.2, Identification of Data Needs and 3.12.3, Ongoing Studies.*

Page 87, line 17, page 90 line 14, page, 91, line 17, page 94, line 27. Cavalli et al. not an appropriate primary source for this information.

Page 91, lines 21, 24, 27, and 31. (EPA 2006c) is missing from the Reference section.

CHAPTER 7. ANALYTICAL METHODS

This chapter begins with standard language (in bold). Most information should be presented in tabular form.

- *Are you aware of additional methods that can be added to the tables? If so, please provide copies of appropriate references.*

Page 104, section 7.1. This section does not describe the measurement of acrylamide and glycidamide in serum (Doerge et al. 2005b), or DNA adducts (Doerge et al. 2005a) as conducted by Doerge and colleagues. The references have been cited elsewhere in the text.

Page 104, line 32. This paragraph describes GC-MS for analysis of hemoglobin adducts of acrylamide and glycidamide. Most investigators now use LC-MS/MS for analysis of these adducts, e.g. as in Fennell et al. (2003), which is the first publication of this approach.

Fennell, T. R., Snyder, R. W., Krol, W. L. and Sumner, S. C. (2003). Comparison of the hemoglobin adducts formed by administration of N-methylolacrylamide and acrylamide to rats. Toxicol Sci 71:164-75.

- *Have methods been included for measuring key metabolites mentioned previously in the text?*
- *If unique issues related to sampling for the substance exist, have they been adequately addressed in the text? What other discussion should be provided?*
- *For Section 7.3.1, Identification of Data Needs, answer the same questions presented in Section 3.12.2, Identification of Data Needs.*

CHAPTER 8. REGULATIONS AND ADVISORIES

This chapter should present most information in tabular form. Information that is relevant but does not fit conveniently into the tabular format may be described in a brief paragraph. ***NOTE:*** *In the table, only IARC and WHO recommendations are to be included under "International."*

- *Are you aware of other regulations or guidelines that may be appropriate for the table? If so, please provide a copy of the reference.*

No.

CHAPTER 9. REFERENCES

The intent of this section is to provide a reasonably complete list of references, whether cited in the text or not. Every reference cited in the text should appear with an asterisk in the bibliography.

- *Are there additional references that provide new data or are there better studies than those already in the text? If so, please provide a copy of each additional reference.*

Fennell, T. R., Snyder, R. W., Krol, W. L. and Sumner, S. C. (2003). Comparison of the hemoglobin adducts formed by administration of N-methylolacrylamide and acrylamide to rats. Toxicol Sci 71:164-75.

Fennell, T. R., Sumner, S. C., Snyder, R. W., Burgess, J. and Friedman, M. A. (2006). Kinetics of elimination of urinary metabolites of acrylamide in humans. Toxicol Sci 93:256-67.

Ghanayem, B. I., McDaniel, L. P., Churchwell, M. I., Twaddle, N. C., Snyder, R., Fennell, T. R. and Doerge, D. R. (2005). Role of CYP2E1 in the epoxidation of acrylamide to glycidamide and formation of DNA and hemoglobin adducts. Toxicol Sci 88:311-8.

Ghanayem, B. I., Witt, K. L., Kissling, G. E., Tice, R. R. and Recio, L. (2005). Absence of acrylamide-induced genotoxicity in CYP2E1-null mice: evidence consistent with a glycidamide-mediated effect. Mutat Res 578:284-97.

Walker, K., Hattis, D., Russ, A., Sonawane, B. and Ginsberg, G. (2007). Approaches to acrylamide physiologically based toxicokinetic modeling for exploring child-adult dosimetry differences. J Toxicol Environ Health A 70:2033-55.

Review of Unpublished Studies Provided on CD.

1. American Cyanamid Company. 1951. Acute eye, dermal and oral toxicity. Submitted to the U.S. Environmental Protection Agency under TSCA Section 8D. EPA878211662. OTS206055.

This study investigated eye and dermal irriation of acrylamide in rabbits and oral toxicity in mice. The LD_{50} on mice was reported to be 195 mg/kg.

Agreed with the conclusions of the authors.

2. American Cyanamid Company. 1953. Inhalation toxicity supplement to reports dated May 2, 1951 and August 13, 1952. Submitted to the U.S. Environmental Protection Agency under TSCA Section 8D. EPA879211664. OTS206055.

Animals (2 dogs, 7 rats and 7 guinea pigs) were exposed to acrylamide dust by inhalation for 6 hours per day, 5 days, per week for 16 days, with a total of 12 exposure days. The exposure concentration was determined gravimetrically as 0.0156 mg/L.

Four rats died following the first exposure, and symptoms of neurotoxicity were observed on 4^{th} day in survivors. One rat was found dead on the 6^{th} day, one sacrificed on the 8^{th} day, and the remaining rat was found dead on the 9^{th} day.

One dog showed excitability, lack of coordination, and weight loss at the beginning of the second week. After stopping exposure, the dog died on day 15. The remaining dog survived to the end of the exposures, with weight loss, and symptoms of neurotoxicity. No sign of toxicity were observed in guinea pigs. Based on the pathology of the rats and dog that died, the authors concluded that the inhaled acrylamide dust acted as an irritant in the lungs, as well as a neurotoxicant.

3. American Cyanamid Company. 1953. Subacute feeding section I-acrylamide section II-acrylamide with metabolic studies supplement to report dated August 13, 1952. Submitted to the U.S. Environmental Protection Agency under TSCA Section 8D. EPA878211665. OTS206055.

Young male Caworth Farms strain rats were exposed (10 rats per group) to 0, 500, 1000, and 2500 ppm acrylamide in the diet. Toxicity and death was observed between days 7-21. Survivors in the 500 ppm group showed signs of muscle weakness and paralysis, which gradually improved on transfer to control diet. A second study was conducted in which rats were administered 10, 50 and 100 ppm in the diet for up to six weeks. Growth retardation was observed in the 100 ppm group, and no paralysis or weakness was observed in any of the dose groups. Feeding 1000 ppm alanine in the diet to rats with 300 ppm acrylamide did not change the growth retardation or symptoms of toxicity with acrylamide alone.

Agreed with the conclusions of the authors

4. American Cyanamid Company. 1953. Chronic oral administration-dogs oral administration and neurohistology-dogs supplement to reports dated August 13, 1952 and May 8, 1953. Submitted to the U.S. Environmental Protection Agency under TSCA Section 8D. EPA878211666. OTS206055.

Copy provided too poor to review adequately

5. American Cyanamid Company. 1954. Chronic inhalation exposure-acrylamide. Submitted to the U.S. Environmental Protection Agency under TSCA Section 8D. EPA878211670. OTS206055.

Four cats (3 females and 1 male) were exposed to acrylamide vapor at a concentration of 1.65 ppm, 6 hours per day, 5 days per week for 3 months. Four cats (3 males and 1 female) were exposed to room air alone. The atmosphere concentration of acrylamide was determined by drawing air through water filled impingers, and analyzing for acrylamide colorimetrically, with a separate verification by GC analysis. No toxicity was observed in the cats. Two cats exposed to acrylamide were reported to have kittens during the course of the study, and the kittens were exposed without signs of toxicity.

The study design with unbalanced numbers of males and females in the control and treated groups appears unusual. Two of the cats were pregnant during the course of the exposures.

6. American Cyanamid Company. 1959. Acrylamide toxicity: Effect of polyvinylpyrrolidone (PVP). Submitted to the U.S. Environmental Protection Agency under TSCA Section 8D. EPA878211673. OTS206055.

The document provided was a single page of a report, indicating that polyvinylpyrrolidone (PVP) administered in combination with acrylamide orally to rats does not protect against the toxicity of acrylamide. It also reported that PVP administered to rabbits in combination with acrylamide dermally did no protect against the toxicity of acrylamide. No specific study data was reported.

7. American Cyanamid Company. 1973. Range finding studies single oral single dermal (FHSA) single inhalation. Submitted to the U.S. Environmental Protection Agency under TSCA Section 8D. EPA878211677. OTS206055.

This document contained summary tables of mortality and effects of oral administration to male Harlan Wistar rats at 50, 100, 200, 400, and 800 mg/kg, dermal administration to male albino rabbits at 200, 400,

and 800 mg/kg on intact skin, and 200 and 400 mg/kg on abraded skin. For oral administration to rats, an LD50 value of 294 mg/kg was reported. For rabbits, an LD50 value of 252 mg/kg was reported, although it was not specified whether this applied to the abraded skin, the non abraded skin or all of the animals. For inhalation, 6 female rats were exposed to 2.07 mg/L acrylamide as an aerosol in distilled water. All rats survived the inhalation exposure. The duration of exposure was not specified.

8. American Cyanamid Company. 1977. Limited release toxicity tests. Submitted to the U.S. Environmental Protection Agency under TSCA Section 8D. EPA878211678. OTS206055.

This document contained summary tables of the mortality of male Sprague Dawley rats administered 50% acrylamide solution orally at doses of 0.5, 0.63, 0.79 and 1.0 mL/kg orally, or male New Zealand White Albino rabbits administered 50% acrylamide solution dermally at doses of 1.4, 1.7, 2.2 and 2.8 mL/kg. For oral administration to rats, an LD50 value of 0.75 mL/kg was reported. For rabbits, an LD50 value of 1.68 mL/kg was reported

9. American Cyanamid Company. 1979. A fetal toxicity study of acrylamide in rats. Submitted to the U.S. Environmental Protection Agency under TSCA Section 8D. EPA878211679. OTS206055.

Acrylamide was administered in the diet to female Sprague Dawley rats (20 per group) at doses of 25 and 50 ppm for 2 weeks prior to mating and for 19 days of gestation. Controls received untreated diet. After weaning (Day 21) 4 pups (2 per sex) were removed for histopathology. Body weights in the high dose females were slightly decreased in the premating exposure period. The authors reported that mortality, body weight, food consumption, mating and pregnancy indices, litter and offspring data and gross postmortem observations did not reveal any alterations attributable to acrylamide administration.

A separate pathology report of changes in the nervous system from the pups was also included. Samples were obtained from the central and peripheral nervous system. The authors reported no evidence of a major teratogenic effect of acrylamide, some treated animals showed scattered nerve fiber degeneration in the sciatic and optic nerves, and nerve fibers from treated animals were more susceptible to preparative artifact.

No analysis to verify the concentration or stability of acrylamide in the diet was reported.

10. American Cyanamid Company. 1991. 28-Day subchronic dose toxicity study in rats. Submitted to the U.S. Environmental Protection Agency under TSCA Section 8D. OTS0533854.

Acrylamide was administered in drinking water to male and female Fischer 344 rats at 0, 1.4, 4.1, 12, 19, and 25 mg/kg/day (males) and 0, 1.3, 4.3, 9.0, 19, and 24 mg/kg/day for 28 days. Blood was collected on day 14 by eye bleeding and at study termination from the aorta. Hormone measurements were made (testosterone, T3, T4, TSH, prolactin). Mortality was observed in the highest dose group on the 4^{th} week. Body weight decreases were observed in the two highest dose groups. Food consumption was lower in the top dose group (females) and the three top dose groups (males). Peripheral neurotoxicity was observed in the two top dose groups. Testicular atrophy and/or atrophy of the seminal vesicle was observed in the two top dose groups. Serum testosterone was significantly lower in these groups compared with the controls. Prolactin concentrations in males were significantly decreased in the two high doses (males) at 14 days, and remained below the controls at 28 days. The results were not conclusive due to wide variation in group means and large standard deviations. Differences observed in TSH, T3 and T4 levels were not consistently associated with acrylamide administered or at 14 days vs 28 days, although the authors suggested that random sampling over a 6 hour day and the pulsatile release of hormones may have contributed to variability and differences between the two time points. The authors indicated that dose levels of 4.1 and 4.3 mg/kg were apparent no-effect levels for male and females, respectively.

The authors reported loss of the acrylamide in the drinking water solutions during the first few weeks of exposure, and the doses administered are estimates. Water containing acrylamide was presented weekly, and concentrations were stable on days 1-2, decreased slightly between days 2 and 5, and dropped dramatically between days 5 and 7. The behavior of the concentrations on week 3 was used to extrapolate to the other weeks and estimate the dose administered.

In general agreed with the conclusions of the authors.

11. Dow Chemical Company. 1957. Results of toxicological tests on acrylamide with cover letter. Submitted to the U.S. Environmental Protection Agency under TSCA Section 8D. EPA878210955. OTS206135.

This report contains a synopsis of LD_{50} values in rat guinea pig and rabbit, characterizes acrylamide as having "high acute toxicity" and describes precautions for safe handling: avoidance of ingestion, skin contact, breathing of vapor or dust, the need for appropriate labeling to avoid accidental swallowing, the use of personal protective equipment, including safety glasses, protective clothing.

There are response remarks included in the tables, with such comments as "slight initial weight loss", or "very slight initial weight loss", however, no details of the number of animals affected, the extent of the weight loss, or whether it was statistically significant was not reported.

The report did not mention the use of gloves for protection against dermal exposure.

12. Dow Chemical Company. 1981. Effects of acrylamide monomer on sensory thresholds in monkeys Sept. 15, 1979 to Sept. 18, 1981. Submitted to the U.S. Environmental Protection Agency under TSCA Section 8D. EPA878210964. OTS206135.

This report describes a study to evaluate the effect of acrylamide administration on the sensory thresholds (vibration and electrical) in monkeys. Four female monkeys were orally administered 10 mg/kg /day acrylamide, 5 days per week in orange juice. Body weight decrease was noted. Vibration sensitivity was reduced, whereas electrical sensitivity remained unchanged. Vibration sensitivity remained reduced for several weeks following dosing.

13. Gorzinski SJ, Morden DC, Albee RR, et al. 1979. Results of palatability (12-day) and tolerance (21-day) studies on acrylamide monomer administered in the drinking water to rats. Dow Chemical Company. Submitted to the U.S. Environmental Protection Agency under TSCA Section 8D. EPA878210959. OTS206135.

The copy provided was of a poor quality, and could not read enough to reliably review.

14. Johnson KA, Beyer JE, Bell TJ, et al. 1984. Acrylamide: A two-year drinking water chronic toxicity-oncogenicity study in Fischer 344 rats. American Cyanamid Company. Dow Chemical U.S.A. Nalco Chemical Company. The Standard Oil Company. Submitted to the U.S. Environmental Protection Agency under TSCA Section 4. OTS0507273.

Male and female Fischer 344 rats were administered drinking water to deliver 0, 0.1, 1, 0.5, or 2.0 mg/kg/day for 2 years (60/sex/dose). Drinking water was changed every 4 days, and monitored for stability. Parameters measured were: mortality, clinical signs, body weights, food consumption, water consumption, clinical chemistry, hematology, urinalysis, gross pathology, organ weights, and histopathology. Increased mortality was observed at 2.0 mg/kg/day, particularly in females, beginning at the 21st month. Degeneration of the peripheral nerves was noted histopathologically. Increased incidence of tumors was noted at 2.0 mg/kg/day: in males, scrotal mesotheliomas, and thyroid gland – follicular epithelium. At 0.5 mg/kg/day in males, the only tumor increased was the scrotal mesotheliomas. In females at 2.0 mg/kg/day, there was an increased increase in mammary gland tumors (benign and malignant), cns tumors (malignant), thyroid follicular epithelium (combined benign and malignant), mouth (benign), uterus (malignant), and clitoral gland (benign).

No Figures and Tables were provided in the document for review, although the table of contents indicated the presence of Figures 1-4 and Tables 1-26.

15. Johnson KA, Beyer JE, Bell TJ, et al. 1985. Acrylamide: A two-year drinking water chronic toxicity-oncogenicity study in Fischer 344 rats. Electron microscopy portion. Submitted to the U.S. Environmental Protection Agency under TSCA Section 8D. EPA878216184. OTS0506849.

This is an adjunct study to the Johnson et al. 2-year bioassay, focusing on the effect of acrylamide exposure on the tibial nerve examined by light and electron microscopy. Doses were 0, 0.1, 1, 0.5, or 2.0

mg/kg/day for up to 2 years. Three rats per sex per dose group were sacrificed at 3, 6, 12, 18, and 24 months. The only statistical analysis reported was for body weight. The ultrastructural data was presented as percentage of fibers with effects for each dose group. It is not possible to evaluate the number of animals per group with effects. Early degenerative changes were noted at 2 mg/kg/day at 3 and 6 months by electron microscopy, and these continued and became more severe by 12 months. Spontaneous degenerative changes by 18 and 24 months obscured possible effects attributable to acrylamide at the ultrastructural level.

16. Marty J, P, Vincent CM. 1998. In vitro percutaneous absorption of acrylamide across human skin. Faculty of Pharmacy. University of Paris Sud in the Research Unit in Dermopharmacology and Cosmetology.

This report described an investigation of the diffusion of acrylamide across human skin. 2,3-^{14}C acrylamide was added to a 2% polyacrylamide gel formulation (polyacrylamide 1828 MP as a 2% aqueous gel) to give a concentration of 1.28 and 2 ppm acrylamide. Percutaneous absorption was measured in glass Franz diffusion cells, at 37oC. A small amount of gel formulation was applied to the skin surface without occlusion. The amount of radioactivity crossing the skin was used to calculate absorption. Abdominal human skin was obtained from plastic surgery, and dermatomed to 450 µm. The area available for diffusion was 1.76 cm^2, and the receiver cell volume was 6 mL. The receiver fluid was a phosphate buffer with 0.1% azide as preservative. The receiver fluid was collected at 6, 12, and 24 hours after addition of acrylamide/gel. The skin was washed with saline and dermis and epidermis was separated at 24 hours. Samples were analyzed by liquid scintillation counting for determination of radioactivity. Of the applied acrylamide, 28% and 22% of the radioactivity crossed the skin at 1.28 and 2 ppm, respectively. Little acrylamide accumulated in the epidermis or the dermis. The total absorption represented 33% and 27% at 1.28 and 2 ppm, respectively. While the authors concluded that acrylamide diffuses rapidly, and equilibrium was achieved of the penetration was achieved after 6 hours, the amount of radioactivity continued to increase substantially between 6 hours and 24 hours.

A limitation of the study is that radioactivity was used to quantitate transfer across skin. The nature of the radioactivity (unchanged acrylamide, or metabolites) was not determined.

17. Ramsey JC, Young JD, Gorzinski SJ. 1984. Acrylamide: Toxicodynamics in rats. Submitted to the U.S. Environmental Protection Agency under TSCA Section 4. OTS0507270.

This report describes a study of the fate of [1,3-^{14}C] acrylamide administered by i.v. oral or dermal routes to male Fischer 344 rats. For i.v. administration and blood sampling, rats were implanted with jugular vein cannulas. For i.v. administration a solution was prepared in sterile saline, for gavage administration in distilled water, and for dermal administration in a 1% solution in Triton X-45 surfactant. The dose administered was determined by differential weighing of the dose syringe before and after dosing. The methods section does not clearly describe the details of the study, and it is only with the results that the some of the study details become clear. It was not recorded in the report how urine, feces, and expired CO_2 were collected, although results are presented. It is not reported how the dermal dose was applied, whether the skin was shaved, or occluded after application. It appears that a single dose was administered by i.v. injection at a dose of 2 or 100 mg/kg. Urine was the primary route of excretion (67 and 62% of the dose at 2 and 100 mg/kg, respectively). CO_2 accounted for 4.2 and 3.0 % of the dose at 2 and 100 mg/kg, respectively. Approximately 13% and 14% was recovered in the carcass, and the overall recovery was approximately 88% and 85% at 2 and 100 mg/kg, respectively. Little radioactivity was recovered in the skin, cage wash or feces.

A group of rats was administered a dose of ^{14}C acrylamide 50 mg/kg i.v., and radioactivity was determined in blood, plasma RBCs, and tissues at 0, 6, 12, 18, 24, and 48 hours following dosing. Radioactivity in plasma dropped quickly, whereas radioactivity in RBCs persisted.

Groups of 3 rats were administered ^{14}C acrylamide by i.v. injection or dermal application at doses of 2 and 50 mg/kg. Blood samples were analyzed periodically for radioactivity, and by GC/MS (details not provided). Radioactivity was cleared from the plasma samples in a biphasic manner with both dose routes with an initial half life of 2 hours and a terminal half life of 10 hours. The authors reported that the initial half life represented clearance of the parent compound whereas the terminal half life represented clearance of metabolites. The authors reported that the relative area under the curve for dermal vs. i.v. administration suggested approximately 25 % of the dermally administered dose was taken up. No details of how the pharmacokinetic analysis was conducted were provided.

Oral multiple-dose studies were conducted in which ^{14}C acrylamide was administered at daily doses of 0.05 or 30 mg/kg for 13 days. Excretion of urinary radioactivity was tabulated. The concentration of radioactivity in RBCs reached a plateau at 4-5 days. The daily excretion of radioactivity in urine accounted for approximately 60 % of the daily dose

While the report describes the major urinary metabolite of acrylamide as N-acetyl-S-(3-amino-3-oxopropyl)cysteine, with less than 5% present as acrylamide after oral intravenous or percutaneous absorption, no details of how this determination was made were presented.

18. Rohm and Haas Company. 1975. Acrylamide and methacrylamide. Subchronic percutaneous toxicity study in new-born rabbits. Submitted to the U.S. Environmental Protection Agency under TSCA Section 8D. OTS205982.

Newborn rabbits were administered 0, 0.5, 5.0, or 50 mg/kg/day for 5 weeks. At 5 weeks, one half of the rabbits were killed, and the remaining rabbits in the high dose group were removed from treatment and observed for 7 weeks. Acrylamide was administered in an aqueous solution in containing 0.5 % Triton X 165. The daily dose was divided in three portions, administered at least 3 hours apart. The dose was applied to the back, and rubbed into the skin. The fur was clipped from the rabbits back at least twice weekly.

The two lower dose groups and controls were continued on treatment for the remaining 7 weeks. Neurotoxicity was reported in the high dose group starting at day 24. By day 35, 17 of 23 rabbits were affected. No neurotoxicity was observed in the lower dose groups. Neurotoxic signs decreased during the 7-week recovery period. Three rabbits died in the high dose group, one each in weeks 4, 6, and 7. Severe prurulent bronchopheumonia and pleuritis was observed.

Significant differences in body weight, and organ weights and relative organ weight in the high-dose rabbits sacrificed at 5 and 12 weeks were presented in tables, but none of the findings were considered to be related to the administration of acrylamide by the authors. There was no consideration of gender of the rabbits in the report.

19. Union Carbide Corporation. 1947. Range finding tests on acrylamide with cover letter. Submitted to the U.S. Environmental Protection Agency under TSCA Section 8D. EPA878212200. OTS206058.

This report contains a summary of range finding tests on acrylamide for toxicity: Oral LD_{50} in male rats dosed with a 1% solution of acrylamide in water of 0.316 g/kg; an effort to generate an inhalation exposure aborted because of polymerization; and irritation tests in rabbits, producing no effects with a 40% solution of acrylamide in acetone applied to the rabbit belly, and no damage to 4 of 5 rabbit eyes, and minimal corneal damage in one rabbit administered a 40% solution in propylene glycol.

No indication of the total dose administered to the rabbits was provided.

The author concluded that "Aside from the dangers from ingestion, this compound should not present any great hazard to the persons handling it, unless it should prove to be a powerful sensitizer". Clearly our understanding has changed since 1947, and we know that dermal and inhalation exposure can be significant.

SUMMARY COMMENTS RECEIVED FROM

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Toxicological Profile on Acrylamide
A Task Order – 200-2009-F-30392
Klaunig – Reviewer

Overview

Overall this is an excellent toxicological profile for acrylamide draft. The author(s) of this draft should be commended for collecting all of the relevant publications and salient documents relating to acrylamide health effects. In addition, the document presents the proper review of the literature without bias. In response to the guidelines for peer review of ATSDR Toxicological Profiles, the following critique is noted.

Child health & developmental effects

Child health & developmental effects data relevant to acrylamide has been discussed in the profile. This reviewer is not aware of any additional information relating to this topic that has not been presented in the draft document . The potential effects of acrylamide on offspring from exposure to parents and any indirect effects on the fetus have been discussed in the document. This reviewer is not aware of any other relevant references regarding childhood developmental effects of acrylamide that are not included in the document.

Chapter 1 – Public Health Statement

The chapter is factorial in presentation and presents the information that an average citizen can understand in a non-technical manner. No additional or alternate wording is suggested by this reviewer. The answers to the questions posed and the summary statements are appropriate to address the concerns to the lay public based upon the information that we know about acrylamide. There appears to be no weak sections in this document. Any scientific terms that are used are well defined and no additional definition is needed for this section. One comment may be that under the question 1.3, how might I be exposed to acrylamide? Occupational exposure may occur this is in particular in scientific labs as well as those involved in the manufacture or utilization of acrylamide in various techniques. In section 1.4, how can acrylamide enter or leave the body? There is a potential for skin exposure in occupational settings. Therefore, that may be commented on and as far as leaving the body it is important to note that there may be some in fecal excretion as well as urine and breast milk.

Chapter 2 - Relevance to Public Health

The presentation of this section discusses the significant existing toxicology data, specifically with regard to human health for acrylamide. The effects known to occur in humans are adequately and correctly presented and the references with regard to human exposure of acrylamide appear to be complete. The

discussion of the MRLs for acrylamide via the oral route of acute and intermediate and chronic exposure are presented adequately. Further discussion of the uncertainty factors utilized and how these uncertainty factors or at least a reference to decision-making on these uncertainty factors in the generation of the MRL should be incorporated into the document. The exposure conditions that are referenced are properly detailed and overall this section is well written.

Chapter 3 – Health Effects

The health effects by acrylamide are discussed adequately. The discussion is based upon route of exposure and the resulting toxic effects seen via the various routes of exposure. The author(s) of this document does a very good job of presenting both positive as well as negative data seen in the literature with acrylamide. When data is missing or inadequate, this is noted in the section appropriately. The author(s) should be especially commended for providing an extremely unbiased presentation of the occupational and epidemiological data that is available for acrylamide. The author(s) has presented the salient aspects of each study reported without placing any personal judgment on the results. This was done very well. Where appropriate, the NOAELs and LOAELs were identified and noted in each study. The author(s) used these designations judiciously and defined the value as well as the toxicological endpoint in each case. No changes are suggested for the quality of human studies noted nor the quality of the animal studies noted. One note for consideration would be a small section describing the hemoglobin adducts and the correlation of the hemoglobin adducts with acrylamide exposure. These hemoglobin adducts have been used extensively as a way of detecting acrylamide exposure and the results and the measurements of these as well as the correlation back to actual exposure to acrylamide for pharmacokinetic information has been confusing in the scientific field. It would benefit the document if this aspect of biomarkers (hemoglobin adducts of acrylamide and glycidamid) could be further discussed. A spot for this might be in 3.5.1 where pharmacokinetic mechanisms are discussed .

Levels of Significant Exposure Tables & Figures

The levels of significant exposure tables and figures which summarize the health effects of acrylamide are presented in accordance with the ATSDR Toxicological Profile format. The studies identified are the ones that are appropriate and there do not appear to be any studies not listed in the tables and figures that are missing and should be considered for inclusion. As a user of the ATSDR documents, this reviewer always feels that these tables and figures are confusing at best and certainly do not adequately provide a salient overview of the toxicological endpoints and effects of the compound in question. A note to the ATSDR Toxicology Profile document administration is to consider another approach to this possibly through tables that describe within the table itself the information provided. In addition, the table

legends are way too detailed and expansive to be adequately defined. The strengths of the ATSDR Profile Documents are in the information provided and the extensive review of the research that has been performed and published. These tables and figures for this section do little to assist in my opinion the reader. With that comment, it should be noted that in this particular document on acrylamide, the author(s) did an appropriate job for the LSE tables and figures.

3.3 Genotoxicity

The genotoxicity of acrylamide has been studied extensively both *in vivo* and *in vitro* and has been adequately described in the text of this document.

3.4 Toxicokinetics

The toxicokinetics talking about absorption, distribution, metabolism, excretion are adequately described and cites the acrylamide literature. The issue with using acrylamide adducts of hemoglobin and glycidamide adducts of hemoglobin as measurements of exposure may, as noted above, should possibly be incorporated into this section describing the utilization of hemoglobin adducts as well as the discussion of hemoglobin adducts and exposure levels to acrylamide. What this reviewer is requesting is a section detailing more information in one section about the data available on hemoglobin adducts, how it is measured and the sensitivity selectivity of these adducts. The discussion of the absorption, distribution, metabolism, and excretion is done correctly and the important literature is cited in this area. The information on target organ specificity and distribution of acrylamide is correctly referenced and described. Aspects of the toxicokinetics for acrylamide metabolism is noted. The two PB PK models for acrylamide are discussed in detail in this section. For both models, they are correctly noted that their application to human risk assessment requires further detail and definition.

3.5 Mechanism of Action

The mechanisms of action that have been described to acrylamide effects are detailed and are properly characterized based upon their acute versus reproductive and genotoxic effects. Both the aspects of endocrine disruption in animals and humans as well as childhood susceptibility have been defined and the lack of extensive data for both of these topics with acrylamide has been noted in the document.

3.8 The Utilization of Biomarkers

This section again brings into play the work with acrylamide and glycidamide with regard to measuring these compounds and/or looking at the hemoglobin adducts of these compounds. This section should be expanded and further discussion on the linkage between exposure and hemoglobin adduct formation should be enhanced. This is important since hemoglobin adducts are used to measure exposure levels and the document should provide both in text and possibly in the form of a figure the relationship of acrylamide adducts and acrylamide exposure.

3.8 Biomarkers Used To Characterize Effects

Once again while DNA adduct formation is noted other biomarkers including the hemoglobin adducts that may be important should be noted here.

3.9 Interaction With Other Chemicals

One reference is noted with regard to the clastogenic effects of acrylamide in combination with a calcium antagonist. This appears to be the extent of the data available for this particular topic.

3.10 Susceptible Populations

This section discusses the data on acrylamide with regard to potential susceptible populations. It is important to note that the role of cytochrome 2E1 from the rodent data in the formation of DNA reactive metabolite glycidamide is important and that in particular it should be noted that polymorphisms for this particular enzyme are present in humans and therefore there may be a susceptibility based on the over expression or under expression of this particular enzyme as well as detoxification enzymes related to the metabolism of acrylamide. In addition, since glycidamide is a DNA reactive metabolite, differences in human susceptibility with regard to DNA repair and detoxification mechanisms may make individuals or life stages more or less susceptible to the effects of exposure.

3.11 Methods to Reduce Toxic Effects

This section is adequate and provides the necessary information. It should also be noted that these approaches for reducing toxic effects are not unique to acrylamide but tend to be general for organic chemicals (*i.e.,* not a specific acrylamide antidote).

3.12

The discussion of the adequacy of the database appears appropriate. This section with the identification of data needs is well referenced and presented in an adequate manner. The incorporation of the term ongoing studies (page 76, lines 22 and 23 and page 76, lines 32 and 33) needs further definition and expansion in the text. Are the authors referring to ongoing studies that are looking at the carcinogenicity and genotoxicity of acrylamide in various labs? If so, further definition and detail should be incorporated with regard to that documentation rather than just citing the section where to find further information. For example, in section 3.12.3 ongoing studies on carcinogenicity of acrylamide, these studies are complete and the final reports are being put together for presentation in the spring. This is an important issue in that both acrylamide and glycidamide were examined for the first time in a properly performed chronic study at multiple doses and therefore it will be important to incorporate the findings from this work once concluded into future or amended ATSDR documents. This reviewer is requesting that since these ongoing studies are well defined that perhaps that the abstract or sections from the abstract of the studies can be incorporated into this section. Other than that comment, in general the authors have done an adequate job in identifying the needs for further work and where data gaps exist.

The chemical and physical information on acrylamide is adequately provided and also noted in tabular form.

This section is adequate in the definition of hazardous waste sites that may contain or that do contain acrylamide. This reviewer would like to see the location of these three sites besides noted in figure 6-1 also included in the text of this document on the overview. On page 88, line 10, it should be noted that acrylamide is a rodent carcinogen and a potential human carcinogen as mentioned previously in the introductory material otherwise the potential human exposure section is appropriate.

7 Analytical Methods

Quite a bit of work has gone into understanding and developing methodology for measuring acrylamide both in environmental samples and biological materials. This section would benefit the reader if there was an expansion of the details with regard to the methodology used for the acrylamide detection of

biological materials as well as environmental samples. The reviewer is suggesting adding to the paragraph and increasing additional paragraphs on methodology for detecting these samples and the details of the extraction and preparation of the samples for analysis. In addition, a discussion of the benefits and advantages and disadvantages of using the various techniques.

8 Regulations & Advisory Guidelines

The section on regulations and advisory guidelines appears appropriate. It simply reflects the regulatory aspects.

9 References

The reference listing and citations within the text are more than adequate.

Unpublished Studies on Acrylamide

1) American Cyanamid Company – 1951 – Acute Eye Dermal and Oral Toxicity

This study by American Cyanamid Company examined the effects of acrylamide on ocular installation on eye irritation in male albino rabbits. Mild irritation was noted. This study was properly performed using the state of the science at the time and the results and discussion of the results were correctly interpreted.

2) American Cyanamid Company – 1973

In this unpublished study acute exposure to male albino rats dermally exposed to acrylamide was performed. Toxicity to the CNS was reported. This study was properly performed using the state of the science at the time and the results and discussion of the results were correctly interpreted.

3) American Cyanamid Company – 1977 – Limited Release Toxicity Tests

In this study rabbits were used and rabbits were dermally administered acrylamide at a 50% solution. Clinical signs included loss of coordination. The study appears to be properly performed by American Cyanamide and the study was performed using the state of the science at the time and the results and discussion of the results were correctly interpreted.

4) Dow Chemical Company – 1957 – Results of Toxicological Tests on Acrylamide

In this study rabbits were used. Two doses were administered and the rabbits were given 24-hour dermal application of acrylamide. The results of this study appear to be properly performed using the state of the science at the time and the discussion of this study appeared correct.

5) American Cyanamid Company – 1953 A – Inhalation Toxicity Supplement to Reports

In this study dogs, rats and guinea pigs were exposed to acrylamide dust for a 16-day period. Toxicity in the rats included lethality while in the dogs no toxicity was seen during the first week of exposure while other changes were seen in the dogs after a longer exposure. This study was properly performed using the state of the science at the time and the results and discussion of the results were correctly interpreted.

6) Rohmn Haas Company – 1975 – Acrylamide & Methacrylamide Subchronic Toxicity Study in Newborn Rabbits.

Young 36-hour rabbits 24 per group and 36 control animals were treated by dermal application with acrylamide. Doses ranged from .5 to 50 and toxicity was reported. The study appeared to be performed correctly and the discussion of the results of this study appeared to be appropriate.

7) American Cyanamid Company – 1953C – Chronic Oral Administration to Dogs

In this study a male mogral dog was given a single dose of acrylamide and examined for effects after four (4) hours and after 20 hours. A dose of 100 mg/kg was used. An additional dog was administered 100 mg/kg and also accessed after three (3) days post treatment. The first dog was sacrificed after 10 weeks. This study was properly performed using the state of the science at the time and the results and discussion of the results were correctly interpreted.

8) American Cyanamid Company – 1979 – Fetal Toxicity Study Using Sprague-Dawley or SDCD Rats.

The results of this study which examined post-natal growth and mortality until weaning . This study was properly performed using the state of the science at the time and the results and discussion of the results were correctly interpreted.

9) American Cyanamid Company – 1953B – A Sub-acute Feeding Study With Acrylamide

In this case, male albino rats 10 per group were administered acrylamide in the diet. Concentrations ranged from 0 to 2500 ppm. Clinical signs included neurological changes. This study appeared to be performed correctly and was correctly interpreted.

10) American Cyanamid Company – 1954A – Studies Examining the Effect of Acrylamide Via Inhalation to Four Cats for Three Months

Body weights and clinical signs for acrylamide toxicity were examined. The average dose of 1.6 ppm showed no apparent toxicity. This study was correctly performed and also correctly interpreted.

11) Union Carbide Corporation – 1947 – Range Finding Tests on Acrylamide With Cover Letter

This study examined male albino rats given 1% acrylamide in the drinking water. Deaths and behavioral differences were noted in the rats after 24 hours of exposure and the LD50 was determined

to be approximately 300 mg/kg. This study was performed correctly according to the experimental techniques of the day and the interpretation of this study was correctly made.

12) Dow Chemical Company – 1981 - Effects of Acrylamide Monomer on Sensory Thresholds in Monkeys

In this study, adult female pig-tail monkeys were used and given acrylamide administered in fruit juice. Behavioral changes, weight loss and other signs of acrylamide intoxication were noted. This study appeared to be performed correctly and the interpretation in the results was also done correctly.

13) American Cyanamid Company – 1959 – Acrylamide Toxicity Effect of PVP

Clinical signs of peripheral neuropathy were examined in rats treated with acrylamide. Changes in movement and gait were noted. This study was performed correctly and interpreted correctly.

14) American Cyanamid Company – 1991 – 28-Day Sub-chronic Dose Toxicity Study in Rats

In this study F344 rats were given acrylamide in the drinking water at doses from 0 to 25 mg/kg per day in males and 24 mg/kg per day in females This study was properly performed using the state of the science at the time and the results and discussion of the results were correctly interpreted. .

15) Gorzinski, *et al.* - 1979 –

This study was performed for Dow Chemical Company and provides results on 12-day and 21-day studies of acrylamide given in drinking water to rats. Both clinical and histopathological studies were performed in this study. It appeared that the study was properly performed. This was also used as a range-finding study for a subsequent reported study by Burek, *et al.* 1990. This study was properly performed using the state of the science at the time and the results and discussion of the results were correctly interpreted.

16) Johnson, *et al.* – 1984 – Acrylamide, A 2-Year Drinking Chronic Study

In this study using rats, animals were treated at various concentrations of acrylamide for 6, 12 and 18 months of continuous treatment. The study as performed appeared to be appropriate and the discussion of the results of this study in the document was appropriately done.

17) Johnson, *et al.* 1985 – Acrylamide, A 2-Year Drinking Water Chronic Study in Fischer Rats Electron Microscopy

This study was a chronic study performed in rats as noted in the 1984-based study. This study examined the electron microscopy of the lesions and tissues in the chronically exposed rats. This

study was properly performed using the state of the science at the time and the results and discussion of the results were correctly interpreted.

18) American Cyanamid Company – 1952 – Preliminary Patch Tests With Acrylamide in 25 Persons

This study was conducted on the utilization of patch tests in humans to look at effects including inflammation and localized irritation. This study was properly performed using the state of the science at the time and the results and discussion of the results were correctly interpreted.

19) American Cyanamid Company – 1954B – Summary of Observations Following Single and Repeated Intravenous Doses of Acrylamide to Cats

In this study, cats were given either single- or multiple-intravenous doses and examined for toxicity and behavioral changes following exposure. This study was performed appropriately using the status of science at the time and was correctly interpreted and discussed .

20) American Cyanamid Company – 1954C – Intraperitoneal Injection of Mice

In this study, IP injection of acrylamide into mice was performed to access the IP LD50. The study was performed correctly utilizing the scientific quality at the time. This study was properly performed using the state of the science at the time and the results and discussion of the results were correctly interpreted.

21) American Cyanamid Company – 1956 – Chronic Toxicity Acrylamide

In this study, acrylamide demonomer versus a polymer acrylamide residual material was examined and evaluated for chronic toxicity in rodents. This study was performed correctly utilizing the state of the science at the time and the study was correctly discussed and interpreted.

22) American Cyanamid Company – 1963A – Peripheral Neuropathy in Rats Fed Acrylamide

In this study, the toxic effect of acrylamide on the peripheral neuropathy was accessed. This study was performed correctly and appropriately using the quality and state of the science at the time it was performed. This study was properly performed using the state of the science at the time and the results and discussion of the results were correctly interpreted.

23) American Cyanamid Company – 1963B - Histopathology of the Nervous System of Rats Fed Acryalamide

In this study, acrylamide at a dose of .05% was given orally to rats for a total of two (2) weeks. The study was utilized to examine histopathology changes in the nervous tissue of the rats. The study was performed appropriately using the state of the science at the time it was performed. This study was properly performed using the state of the science at the time and the results and discussion of the results were correctly interpreted.

24) Beyer, *et al.* – 1957 – Electon Microscopic Examination of the Nerve From Two Acrylamide Treated Monkeys

This is a study performed at Dow Chemical Company that was submitted to the EPA for evaluation. In this study monkeys were treated with acrylamide and examined and the nervous system, in particular, the sural nerve was examined utilizing electron microscopy. These changes were correlated with impairment studies with regard to electrical signal. The study was performed using the state of the science at the time and has been duly and correctly discussed .

25) Dow Chemical Company – 1975A – Acute Toxicity Study of Acrylamide

In this study the effects of acute toxicological properties of acrylamide in particular a 50% aqueous solution was examined and documents submitted to the EPA for evaluation. The study appeared to be appropriately performed using the status of the scientific approach at the time and was appropriately noted within the draft document.

26) Dow Chemical Company – 1975B – Determination of Nerve Function In Acrylamide Employees

In this study the effects of neurofunction, in particular neurotransmission, in those employees working with acrylamide versus a control group appropriately chosen was performed. The study appeared to be appropriate utilizing the state of the science at the time and was included and discussed correctly.

27) Dupont Chemical Company – 1956 – Acute Oral Toxicity Testing of Acrylamide

In this study from Dupont Chemical Company, the acute oral testing was performed in rats for acrylamide. This study was properly performed using the state of the science at the time and the results and discussion of the results were correctly interpreted.

28) Gorzinski, *et al.* – 1984 – Acrylamide

This study looked at the effects of interim sacrifices during a two-year drinking water chronic toxicity study in F344 rats. Multiple support was performed from several producers of acrylamide. The study was performed correctly and was interpreted correctly.

29) Marty, J. P. and Vincene, C.M. – 1998 –

This is a non-refereed study on the *in vitro* absorption of acrylamide in human skin material submitted by the faculty of pharmacy at the University of Paris. This study was performed appropriately utilizing *in vitro* techniques and utilizing science quality at the time. The study has been appropriately performed and discussed .

30) Monsanto Company – 1954A – Acrylamide Skin & Eye Irritation Studies

In this report irritation studies with acrylamide were examined in rabbits. This study was performed using the appropriate procedures and scientific approaches of the time and was correctly discussed.

31) Monsanto Company – 1954B – Skin & Eye Irritation Studies with Acrylamide

In this study additional parameters were measured examining acrylamide effects on skin and eye irritation. It was performed correctly utilizing the state of the science at the time and was correctly discussed.

32) Nalco – 1977 – Acrylamide in Exhaust

This is a report from the Nalco Chemical Company submitted to the U.S. EPA examining the effects of exhaust sampling data and measuring acrylamide. This study was properly performed using the state of the science at the time and the results and discussion of the results were correctly interpreted.

33) Ramses, J.C., *et al*. – 1984 – Acrylamide Toxicodynamics in Rats

In this study rats were examined for the absorption, distribution, elimination and metabolism of acrylamide following acute exposure. This study was properly performed using the state of the science at the time and the results and discussion of the results were correctly interpreted. .

34) Rodwell, D.E. – 1990 – Neurotoxicity Study

In this study by Dean Rodwell, examination of observational neurotoxicity in rats treated with acrylamide was examined. In these animals, acrylamide showed a dose response of change in observational neurotoxicity. This study was performed properly and was properly discussed.

35) Stockhausen - Skin Sensitization of Acrylamide

In this study, acrylamide at a concentration of 50% was painted onto the skin of guinea pigs and the effects of skin sensitization was examined. This study was performed adequately using the state of the science at the time and was properly discussed.

36) Union Carbide Corporation – 1957 – Range Finding Studies With Acrylamide

In this document the range finding tests for the toxicity of acrylamide in rodents was examined. This study was performed appropriately using the state of science at the time and was properly interpreted.

SECTION B

ADDITIONAL REFERENCES AND DATA SUBMITTED BY THE PEER REVIEWERS

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